were funded by National Science Foundation grant GS-35501 to the University of Michigan with M.C.W. as principal investigator, and supplemented in 1973 by support from the Instituto Nacional de Antropologia e Historia, Mexico. I thank J. B. Griffin and K. V. Flannery, University of Michigan; R. E. Blanton, City University of New York; and I. Bernal, M. Esparza, L. Gamio, and E. Valencia, INAH, for their cooperation. I also thank those who assisted with the excavations and analysis: E. Anderson, E. Abbink, J. Appel, W. Autry, B. Byland, C. Cameron, J. Nowack, E. Redmond, C. Spencer (who also helped with the statistics), J. Stein, M. Whalen, R. G. Wilkinson, D. Wilson, D. F. Wilson, J. F. Zeitlin, and R. Zeitlin. I also thank P. Armillas, W. T. Sanders, E. Bejarano, M. Frangipane, M. Gandara, J. Gussinyer, L. Manzanilla, A. Martinez, L. Ochoa, A. Oliveros, L. Rodrigo, T. Rojas, and O. Schondube.

Multienzyme Systems of DNA Replication

Proteins required for chromosome replication are resolved with the aid of a simple viral DNA template.

Randy Schekman, Alan Weiner, Arthur Kornberg

Template direction of nucleic acid synthesis was seen first in DNA synthesis catalyzed by DNA polymerase and only later was observed in RNA synthesis by RNA polymerase. Nevertheless, work on RNA polymerase during the last 10 years has explained key physiological features of transcription (*1*), whereas the reconstruction in vitro of replication by DNA polymerase action alone has remained inadequate. The basis for this discrepancy in progress is rooted in a biochemical fortuity. By means of enzyme fractionation procedures, RNA polymerase was isolated from cell extracts as a large multisubunit transcriptase. Isolated DNA polymerase, on the other hand, is only one component of a multienzyme DNA replicase. The purpose of this article is to describe the first stage in our efforts to identify and reassemble the pieces of this multienzyme system.

Polymerase actions. The basic elements in synthesis of a nucleic acid are the same whether the chain produced is DNA or RNA, and whether template directions are taken from DNA or RNA (*2*). Elongation of a 3′,5′-phosphodiester-linked polynucleotide invariably has these features: (i) Substrates are a 3′-hydroxyl terminated chain (primer terminus) and a 5′-nucleoside triphosphate. Nucleophilic attack by the primer terminus adds a 5′-nucleoside monophosphate to the chain. (ii) Selection of the specific triphosphate depends on its forming a Watson-Crick base pair with the template. (iii) Growth of the chain is necessarily in a 5′ → 3′ direction, antiparallel to the template.

Among the more than 20 DNA polymerases of viral, bacterial, and animal origin isolated to date, none can start a chain in vitro. This feature distinguishes DNA polymerases most clearly from RNA polymerases. The essence of transcription is the highly selective copying of passages from the chromosome record, and the capacity of RNA polymerases to start chains at defined "promoter" sequences is a prominent part of its function. Thus, DNA polymerases, remarkable for their error-free copying of the entire chromosome, are apparently blind to initiation signals, including the one promoting the origin of a replication cycle. Until recently the enzymatic mechanism of starting DNA chains remained an enigma.

The chain initiation enigma. Two kinds of DNA chain starts need to be considered: (i) initiation of chromosome replication at its unique origin, and (ii) initiation of the short replication fragments that are synthesized discontinuously (Okazaki pieces) at the growing fork or nascent region of the chromosome. This distinction is indicated by genetic and biochemical experiments. Several *Escherichia coli* mutants, thermosensitive in DNA replication [*dna*A and *dna*C (*3*), and, more recently, *dna*H and *dna*I (*4*)], are defective in initiating replication at the chromosome origin. They are unlike other mutants [*dna*B, *dna*E, and *dna*G (*3*)] whose DNA replication stops abruptly when the temperature is raised to a restrictive level; the origin-defective mutants continue DNA synthesis until the chromosome duplication under way is completed. Among the abrupt-stop mutants, *dna*G has been implicated in nascent chain starts (*5*) and *dna*E shows a defect in DNA polymerase III (*6*). Fruitful as these genetic studies are, elucidating the mechanism of chain initiation requires a biochemical approach.

Discovery of these numerous thermosensitive, *E. coli* replication mutants illustrates the multiplicity of gene products needed for chromosome replication. This was anticipated since the phage T4 chromosome, only a twentieth the size of *E. coli*, still induces formation of at least six proteins essential for its replication. We now find from our studies reported below that much of the complexity of the replicative machinery resides in the events of chain initiation. This recent progress is based on two things: the use of small phage chromosomes as probes and the development of a cell extract consisting of soluble enzymes and capable of phage chromosome replication.

Phage probes and soluble enzymes. Attempts to understand how DNA chains are started and elongated had been frustrated by the use of large chromosomes, such as those of bacteria and medium-sized phages, and, in addition, by the fragmentation of the multienzyme replication system. Enzymological studies have been greatly aided by the use of the single-stranded (SS) circular chromosome of the small DNA phages (*2*).

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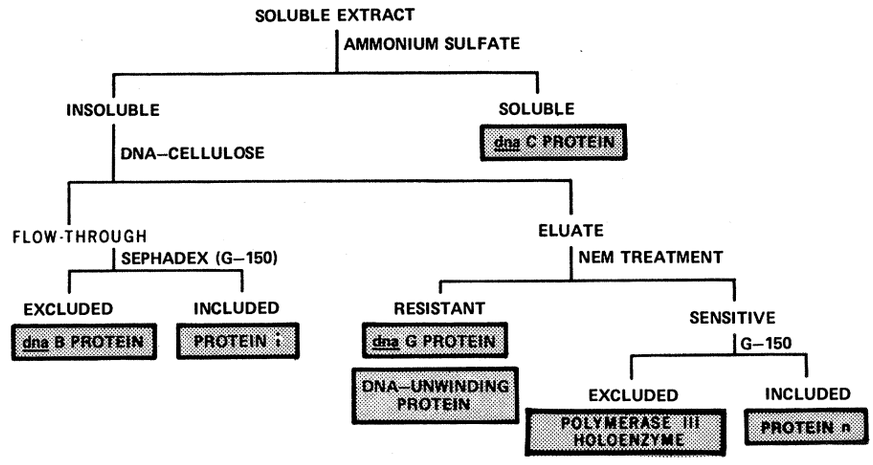

Fig. 1. Scheme for resolution of the øX174 enzymes. Soluble extracts were prepared from *E. coli* H560 as described before (*8, 9*). The outlined procedure (*22*) of subdividing the essential components provided assays for purifying each of the proteins.

The filamentous phage M13 (also fl and fd) and polyhedral phage øX174 (also S13 and G4) offer several crucial advantages. (i) The tiny viral chromosome contains only eight to ten genes, devoted mostly to coat proteins and assembly and so must rely on the replicative systems of the host cell. Thus, in appropriating host enzymes for its replication, the phage chromosome may illuminate mechanisms by which more complex chromosomes are handled. (ii) The viral chromosome is a relatively simple template molecule preparable in large quantity, is easily purified and characterized, and becomes part of a readily analyzed product. (iii) The initial event upon infection is conversion of a SS circle of DNA to a double-stranded replicative form (RF). Therefore, initiation of a new chain on the viral template in vitro should be less complicated by artifact than with a duplex template. In the latter, endonucleolytic scission of one strand can provide a primer terminus for copying the other strand. (iv) The template may be so small as to contain only one initiation signal.

In a series of investigations in which the bacterial chromosome served as template, DNA replication could be observed only with permeable cells or with membranous, immobilized lysates (*7*). Many valuable insights into the role of external substrates and factors emerged from the studies with these complex subcellular preparations, but resolution of the replicative system into its molecular components was not attained. The choice of a tiny phage chromosome offered not only the practical advantages just enumerated, but, equally important, the psychological impetus needed to undertake the resolution of a complete replication system. It seemed reasonable that the apparently simple copying of a small DNA circle could be attained with purified enzymes and that, without such success, attempts with large chromosomes would be futile. A soluble enzyme system which could be resolved into its components was clearly necessary.

Preparing such a soluble enzyme extract from *E. coli*, which efficiently converts the M13 or øX174 SS to RF, depended on several exacting conditions (*8, 9*): (i) the harvesting of young growing cells without chilling, (ii) the use of gentle lysozyme lysis, with minimal disruption of DNA, and (iii) the removal of virtually all the host DNA with particulate material by sedimentation at high speed. Extracts prepared in this way have since been applied successfully to replicate the larger phage chromosomes of T7 and λ (*10*) and, most recently, the intact, folded *E. coli* chromosome itself (*11*).

RNA priming of DNA synthesis. The ability of RNA polymerases to start new chains, and the capacity of DNA polymerase to extend a polyribonucleotide primer, led us to explore the possibility that a brief RNA transcript might prime DNA synthesis. The effect of rifampicin (a specific inhibitor of RNA polymerase) on the conversion of the M13 viral circle to its duplex form was chosen as a test of this hypothesis. The drug blocked the conversion of M13 DNA both in vivo and in cell extracts (*8, 12*). Rifampicin-resistant RNA polymerase mutants did not show this effect. Additional evidence then indicated that the action of RNA polymerase was needed to provide an RNA primer. A stage of transcription was separable from a subsequent stage of DNA synthesis. A dramatic verification of RNA priming by RNA polymerase action has come with the isolation of an intermediate form in rifampicin-sensitive replication of the DNA plasmid colicin E1,

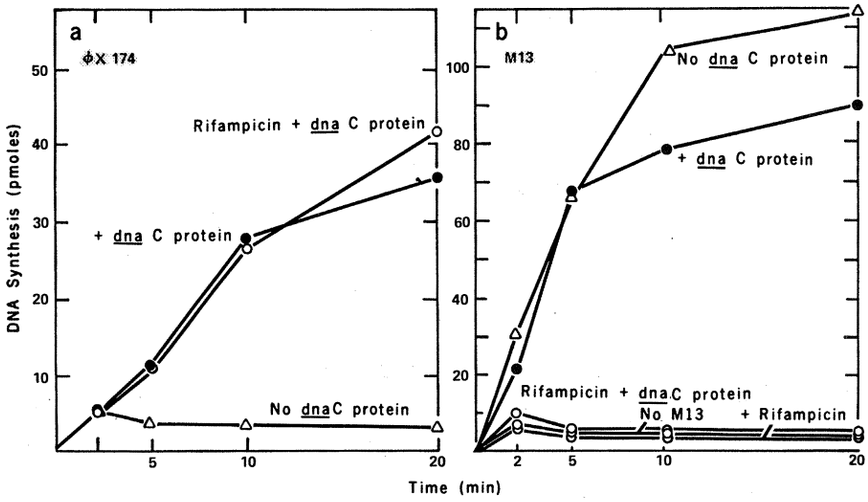

Fig. 2. Partially purified *dna*C protein required for øX174 (a) but not M13 (b) replication. Partially purified *dna*C protein (*22*) was added to a soluble enzyme fraction prepared from *E. coli* PC79 (*dna*C mutant, formerly *dna*D) as described before for wild-type *E. coli* (*8, 9*). Assays (25-μl volume) included 0.3 mg of mutant-extract protein, 0.3 nmole of øX174 or M13 DNA, 20 nmole of ATP, 2.5 nmole each of GTP, UTP, and CTP, 1.25 nmole each of dATP, dGTP, and TTP, 0.45 nmole of [^{32}P]dCTP (400 count/min pmole), 125 nmole of $MgCl_2$, 100 nmole of spermidine hydrochloride; 100 ng of rifampicin and 2.5 μg of *dna*C protein were present where indicated. Incubation was at 30°C.

with the RNA piece still in place (*13*).

The initial choice of M13 replication (SS to RF conversion) as an object for examining rifampicin inhibition was fortunate, inasmuch as a test with øX174 proved negative (*8*). The øX174 result, because it came later, did not discourage our belief in a generalized status for the RNA-priming hypothesis. Instead, the lack of dependence of øX174 DNA synthesis on initiation by RNA polymerase spurred experiments which disclosed RNA priming by a novel RNA synthetic system. Although rifampicin-resistant, the øX174 reaction nevertheless required all four ribonucleoside triphosphates leading to a covalent attachment of RNA to the DNA product (*14, 15*).

With the use of M13, øX174, and, more recently, of G4—which is a øX-174-related phage (*16*)—as probes, we have obtained evidence, presented below, that at least three distinctive replicative systems exist in *E. coli*, each with a characteristic RNA-priming mechanism. Beyond these examples illustrated by the small coliphages, RNA priming of DNA synthesis has been observed in many instances, such as in the nascent replication fragments of the *E. coli* chromosome (*17*) and in animal cells utilizing as template either tumor virus DNA (*18*) or their own DNA (*19*).

Resolution of a multienzyme system. There are two approaches to fractionation of a multienzyme system responsible for a complex reaction. (In this case it is the incorporation of deoxynucleotides into DNA, dependent on M13, øX174, or G4 DNA templates.) One is by complementation of an extract prepared from mutant cells. Assay at an elevated temperature, at which the thermosensitive enzyme is inactive, determines the amount of added wild-type enzyme available. In this way each of the components, for which a mutant deficiency has been identified, can be isolated from wild-type cells. The second approach is direct resolution and reconstitution of fractionated wild-type enzymes, where protein resolving reagents are used to sort out and eventually to isolate each of the components. There are serious limitations to each approach.

Complementation assays have suffered from the following drawbacks. (i) The thermosensitive protein is always present and may be stabilized by a variety of nonspecific compounds. As an example, complementation assays of the *dna*A gene product were confused by the capacity of polyethylene glycol (introduced during a phase partition purification step) to specifically stabilize the mutant extract at a nonpermissive temperature. (ii) If the wild-type protein is normally part of a multienzyme package, the mutant component must be physically replaced. (iii) Multiple deficiencies are often manifested in a mutant extract. Defective enzyme Y may interfere with enzyme Z activity. If Z proves rate-limiting in the assay, Z rather than Y will be purified from wild-type cells. In one instance a *dna*G mutant extract was more deficient in another required activity, DNA polymerase III*, than in the mutant *dna*G protein. Complementation assays with this extract resulted in the mistaken purification of DNA polymerase III*. (iv) When an enzyme has finally been purified, it is unlikely that its mechanism of action can be deduced from complementation of a crude extract. (v) When all the enzymes for which mutants are available have been purified, essential enzymes may still be lacking for which mutants are still unknown. Furthermore, enzymes purified for their capacity to complement a crude extract may depend on activating factors in the extract lacking in a collection of pure enzymes.

There are also serious drawbacks to resolution and reconstitution of a wild-type system. First of all, adequate criteria are necessary to establish an authentic reconstitution. Drug resistance or sensitivity, substrate specificity, or specific enzyme requirements may be used. Once the criteria are available, techniques that give sharp enough resolution are required to provide a reliable assay for each component. Assays are complicated by the need for supplies of each of the numerous purified components, some of which are rather unstable. Order of addition of the individual components to the incubation mixture and their relative amounts may be crucial. Even when all the essential purified components appear to be in hand, the list may lack some that function in the system in vivo or may include some that do not.

Resolution of the øX174 system. Earlier studies showed that the SS to RF reaction with øX174 DNA required many proteins also necessary for *E. coli* chromosome replication. Soluble extracts prepared from temperature-sensitive DNA synthesis mutants *dna*A, B, C, E, and G were defective compared to those from temperature-resistant revertants (*14, 20*). The *dna*B, C, E, and G gene products have been partially purified by means of a complementation assay (*21–23*); however, these proteins are not sufficient for the overall reaction.

We have used a combination of both complementation and total fractionation to resolve and identify the components of the øX174 system. The flow diagram in Fig. 1 indicates the major steps used to segregate the essential components. The purification procedure for a component was reexamined, starting in each case with an independent procedure from the first step to optimize yield and purity.

The *dna*C protein was separated from other essential components by ammonium sulfate fractionation and assayed by complementation of a mutant (*dna*C) extract (*22*). After

Table 1. Enzyme requirements for the reconstituted ϕX174 and G4 reactions. All the purified proteins were assembled in the complete reaction or omitted individually, as indicated. Assays were performed in 25-μl volumes at 30°C for 20 minutes. Components were mixed in the following order: 10 μl of dilution buffer (10 percent sucrose, 50 m*M* tris·HCl, *p*H 7.5, 20 m*M* dithiothreitol, 0.2 mg of bovine serum albumin per milliliter) 0.13 μg of protein n, 0.73 μg of DNA-unwinding protein, a mixture of protein i (40 ng), *dna*B protein (20 ng), *dna*G protein (40 ng) and DNA polymerase III holoenzyme (0.33 μg), and then 2.5 μg of *dna*C protein. A mixture of other components—1.25 nmole each of dATP, dGTP and dCTP (*35*), 0.45 nmole of [^{3}H]TTP (240 count/min pmole), 125 nmole of $MgCl_2$, 20 nmole of ATP, 2.5 nmole each of GTP, UTP, and CTP, 40 nmole of spermidine·HCl, 0.33 nmole of ϕX174 or G4 DNA and 100 ng of rifampicin—was added to start the reaction. The DNA synthesis was measured by incorporation of the labeled deoxynucleotide into an acid-insoluble form.

Omitted item	Reconstitution (% relative to complete reaction*)	
	ϕX174	G4
None (complete*)	100	100
*dna*B protein	1	150
*dna*C protein	7	150
DNA polymerase III holoenzyme	2	12
*dna*G protein	7	13
Protein i	12	130
Protein n	4	150
Spermidine	2	75
Unwinding protein, spermidine	15 1	140 12

* In the complete reactions 120 pmole of deoxynucleotide was incorporated with ϕX174 DNA as template, 42 pmole with G4 DNA, but only 2.5 pmole with M13 DNA; the M13 DNA was used under conditions identical to those used for ϕX174 and G4 DNA's.

Table 2. Ribonucleoside triphosphates required for initiation. Reactions were largely as described in Table 1. Reactions 2 to 5 contained 10 nmole of ATP (purified on Dowex 1X-8) in addition to 2.5 nmole of the other rNTP's (*35*) as indicated. Dilutions were performed as in Fig. 3b, except for reaction 6 which contained a tenfold complement of rNTP's in the dilution mixture. Reactions 7 and 8 were not diluted; a single complement of rNTP's or dNTP's or both was added to start the second-stage reaction. First-stage reactions were incubated for 10 minutes; second-stage reactions (1 to 5) were incubated for 10 minutes; reactions 6 to 8 were incubated for 15 minutes.

Exp. No.	rNTP requirements			DNA synthesis (pmole)
	First-stage*	Tenfold dilution	Second-stage addition	
1	None	+	dNTP's	1.3
2	A	+	dNTP's	2.1
3	A, G	+	dNTP's	6.2
4	A, G, U	+	dNTP's	7.0
5	A, G, U, C	+	dNTP's	16.0
6	None	+	rNTP's + dNTP's	1.1
7	None	None	rNTP's + dNTP's	84.4
8	A, G, U, C	None	dNTP's	110

* rNTP added.

further purification by phosphocellulose chromatography, this fraction serves in rifampicin-resistant synthesis of ϕX174 DNA by the mutant extract but has no influence on M13 DNA synthesis (Fig. 2). Requirement for *dna*C protein was the touchstone for authentic reconstitution in subsequent fractionation. The other components were divided into two groups by passage through a DNA-cellulose column (Fig. 1). At this stage three fractions are needed for ϕX174 DNA synthesis: the fraction unadsorbed to DNA-cellulose, the fraction bound to and eluted from it, and the *dna*C protein fraction.

The fraction unadsorbed to DNA-cellulose was further divided by chromatography on Sephadex G-150 into an excluded and an included fraction. Each of these was then purified by further chromatography to yield, respectively, *dna*B protein (identified by complementation with a *dna*B mutant extract) and protein i (not identified with any of the *dna*A, B, C, E, or G gene products). At this stage, the components required for activity were DNA-cellulose binding fraction, *dna*B protein, protein i, and *dna*C protein.

The fraction adsorbed to, and eluted from, DNA-cellulose was dissected by sensitivity to the sulfhydryl-blocking agent *N*-ethylmaleimide (NEM) and further resolved by chromatography on Sephadex G-150. Excluded from the gel was the NEM-sensitive, DNA polymerase III holoenzyme (a complex containing DNA polymerase III* and copolymerase III* activities, as is discussed below). Included in the gel were three essential proteins. Two were NEM-resistant and separable into *dna*G protein—identified by complementation (*23*)—and the DNA-unwinding protein (*24*); the other was an NEM-sensitive protein (protein n), corresponding to none of the *dna*A, B, C, E, or G gene products.

Properties of the reconstituted ϕX174 system. The seven protein fractions incubated with the four ribo- and four deoxyribonucleoside triphosphates, spermidine, and Mg^{2+} replicated the ϕX174 viral template (Table 1). Each of the purified proteins was required in the reconstituted reaction; omission of any one reduced synthesis by 5- to 50-fold. The extent of dependence on each protein has varied with degrees of cross contamination in different preparations. In no instance could any pair of proteins be omitted, as might be seen in the action of an anti-inhibitor overcoming the effect of an inhibitor. The reaction is resistant to rifampicin. At 30°C, DNA synthesis progressed at a constant rate for about 40 minutes (Fig. 3a) with as much as 50 percent of the DNA copied. The product was RFII, in which a nearly full-length synthetic strand complements the circular viral strand. A small gap between the 3′ and 5′ ends of the synthetic strand remains. Whether this gap is at a unique location relative to the template, as is true for G4 and M13 (see below), is yet to be determined. Conversion of the RFII to the covalently closed duplex RFI requires excision of the RNA-priming fragment at the 5′ end by the 5′ → 3′ exonuclease function of DNA polymerase I coupled with its replicative gap-filling activity, and DNA ligase action to seal the circle (*15*).

Division of the standard reaction into two stages was obtained by dilution. Incubation with the ribonucleoside triphosphates but without deoxynucleoside triphosphates was followed by a tenfold dilution with a buffer containing the latter. Formation of a primed single-strand was measured by its ability to support subsequent DNA synthesis. The rate of the first-stage, priming reaction was constant for 10 minutes then decreased (Fig. 3b), with dilution at any intermediate time reducing the subsequent extent of DNA synthesis. In the absence of new initiations, the second stage was complete within 2 minutes. Optimal reaction in the first stage required all four ribonucleoside triphosphates (Table 2); addition of omitted ribonucleoside triphosphates in the DNA synthesis stage did not suffice. Two-stage reactions were only 20 to 30 percent as efficient as standard reactions. This may be due to degradation of the primer by contaminating ribonucleases in the absence of immediate extension by DNA.

DNA polymerase III holoenzyme (*25, 26*) was absolutely required in

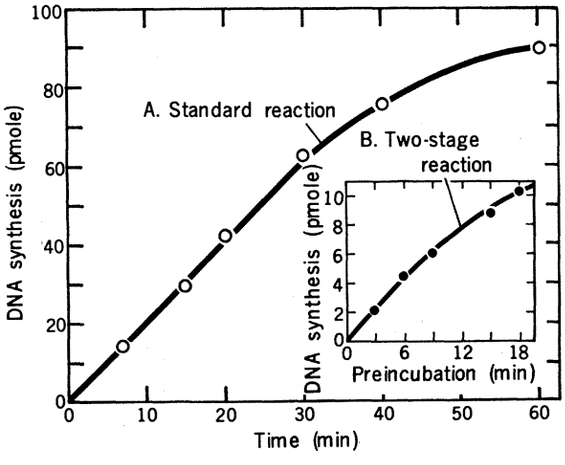

Fig. 3. Time course of standard and two-stage reconstituted ϕX174 reactions. (a) Standard reaction. Components were the same as those described in Table 1. (b) Two-stage reaction. Formation of primed single-strands took place in a reaction mixture of 25 μl lacking only the deoxytriphosphates. After incubation for the indicated times the first stage was stopped by dilution, and the product was measured by its capacity to support DNA synthesis. This was performed by addition of 250 μl of buffer containing the deoxynucleoside triphosphates (4.25 nmole of [^{3}H]TTP, 12.5 nmole each of dATP, dGTP, and dCTP), 1.25 μmole of $MgCl_2$, and 1.7 μg of DNA polymerase III holoenzyme. Diluted reactions were further incubated for 10 minutes.

the reconstituted system (Fig. 4). This multisubunit enzyme contains two *dna*E polypeptides (90,000-dalton chains) and two molecules of copolymerase III* (77,000-dalton chain). The holoenzyme is active on long, SS templates, such as RNA-primed øX174 viral DNA, as well as on templates with short (10 to 20 nucleotides) exposed regions. By contrast, DNA polymerase III can utilize only the latter (*27*).

Either spermidine or the DNA-unwinding protein serve the holoenzyme in its replication (elongation) of a primed, SS template (*28*). However, replication of the øX174 template (unprimed) required both (Fig. 5). Thus both agents may be needed in the initiation stage. Optimal levels of unwinding protein (4 to 7 μg per microgram of DNA template) corresponded to 1 monomer per 8 to 14 nucleotide residues. Binding studies indicate that DNA is saturated at a level of 1 monomer per 8 nucleotides (*29*). The known antagonism of spermidine to unwinding of the DNA duplex needs to be reconciled with its complementary action with unwinding protein in this replication system. These agents behave differently, depending on the secondary structure of the DNA, and may act at different substages of the initiation reaction.

With the components of the øX174 replicative system largely resolved, we have come closer to a direct examination of what each component contributes to the mechanism of the replicative reaction. Its dissection into partial reactions and intermediates should provide important clues. However, it would be a delusion to assume that replicative systems will be free of complexities like those which have bedeviled analysis of the multienzyme systems of transcription and translation for so many years. For example, it is already perplexing that the need for the *dna*A gene product demonstrated in crude extracts (*14*) is no longer manifest in the reconstituted system. Neither protein i nor protein n, both candidates for the *dna*A protein role, were temperature-sensitive when purified from a *dna*A mutant, nor did they complement the mutant extract. Possibly the *dna*A protein serves by counteracting an inhibitor that is present in the crude extract but absent from the reconstituted system. Or perhaps the mutant *dna*A protein itself inhibits the normal reaction in a crude extract.

Resolution and reconstitution of the

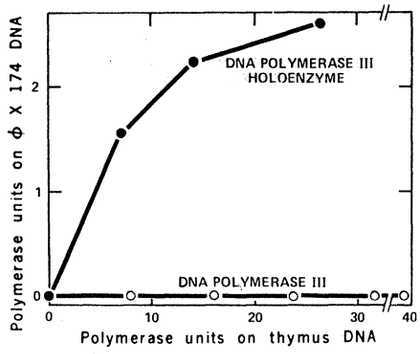

Fig. 4. DNA polymerase III holoenzyme is not replaced by DNA polymerase III in the øX174 reaction. The DNA polymerase III [purified by the method of Kornberg and Gefter (*27*)] and DNA polymerase III holoenzyme (*26*) were compared in reactions on activated calf thymus DNA (*27*) and in the reconstituted øX174 reaction. Reactions (as in Table 1) contained 0.04, 0.08, or 0.16 μg of DNA polymerase III holoenzyme (fraction 4) or 0.04, 0.09, 0.17, 0.34, or 0.68 μg of DNA polymerase III (fraction 4).

G4 system. When the foregoing studies (conversion of SS to RF) were extended to a øX174-like phage, called G4 (*16*), the results were rewarding in an unexpected way. Whereas the template from the closely related phage S13 was indistinguishable from øX174 in its replicative requirements, DNA from the less similar phage G4 was strikingly and gratifyingly simpler (Table 1). Rifampicin-resistant DNA synthesis on a G4 template was achieved merely with *dna*G protein, DNA-unwinding protein (or spermidine), and the DNA polymerase III holoenzyme.

The reaction required ribonucleoside triphosphates and was divisible into two stages, much as the øX174 reaction. The RFII produced in the presence of DNA-unwinding protein contained a nearly full-length complementary strand with a uniquely located gap, as was indicated by analysis of cleavage products generated by the *Eco*RI restriction endonuclease. We have evidence (*23*) suggesting that with this template, *dna*G may be serving as an RNA polymerase to generate the priming fragment for DNA synthesis. The difference in enzyme requirements between øX174 and G4 encourages us to search for other templates which may elicit initiation requirements intermediate in complexity between G4 and øX174.

Resolution and reconstitution of the M13 system. Replication of M13 viral DNA to RF depends on RNA polymerase, and consequently is completely inhibited by rifampicin. The unique location of the small gap in the synthetic complementary strand (*30*) has been taken to indicate the promoter region in the template for the start of RNA

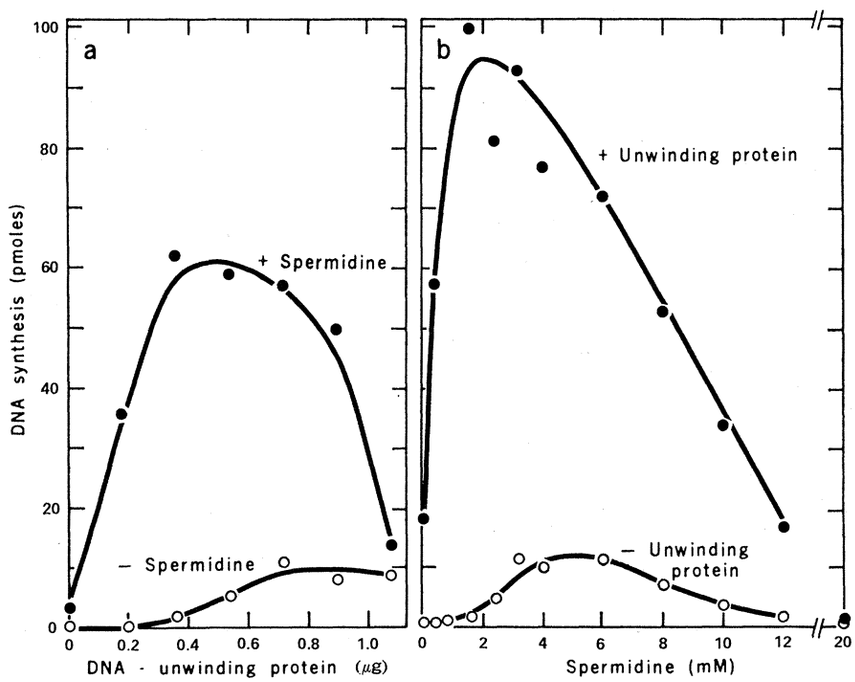

Fig. 5. Requirements for DNA-unwinding protein and spermidine. Reactions were performed as described in Table 1 with DNA-unwinding protein or spermidine concentrations as indicated. (a) Reactions contained 40 nmole of spermidine hydrochloride where indicated. (b) Reactions contained 0.73 μg of DNA-unwinding protein where indicated.

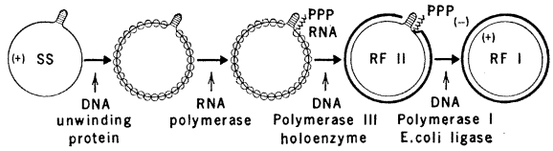

Fig. 6. Scheme for conversion of M13 single-stranded DNA to RFI.

polymerase action (Fig. 6). Unique initiation requires that sufficient DNA-unwinding protein be present to cover the template. Elongation of the chain from the RNA primer depends specifically on DNA polymerase III holoenzyme; and closure of RFII depends on DNA polymerase I and DNA ligase (*18, 28*). This reconstituted M13 system, unlike the crude extract, fails to distinguish M13 from øX174 DNA. This set of purified enzymes supports rifampicin-sensitive, and therefore RNA polymerase–mediated, replication of M13 and øX174 DNA (*28*). Evidently a discriminatory factor present in the crude system has been lost upon resolution and purification of the enzymes (*31*).

Template selection by replicative systems. The three distinctive systems illustrated by the phage template probes are alike in their utilization of the same DNA polymerase III holoenzyme for chain elongation, and DNA polymerase I and DNA ligase for completion and closure of the chain (Table 3). *They are distinguished by their requirements for the initiation event* which primes DNA polymerase action by RNA synthesis. The templates all rely upon the DNA-unwinding protein to mask all but the significant promoter region. In vivo, decapsidation is coupled to replication, and the SS template never enters the cell as such. Therefore, phage coat proteins may function as the DNA-unwinding protein does in vitro.

The G4 template is least demanding; action by the 65,000-dalton *dna*G protein suffices. In this regard, the promoter signals for starting RNA synthesis may resemble those that direct the start of nascent fragments which have been judged to depend only on a few of the *dna* gene products. The øX174 template has far more complex needs for initiation, and the additional proteins may serve to modify the promoter or its recognition for *dna*G protein action. The M13 template is inert with any combination of these proteins (legend to Table 1) and is utilized only by RNA polymerase.

The øX174 template promoter requires a battery of replication proteins including those needed for starting a new cycle of replication of the *E. coli* chromosome. The artifactual priming of øX174 DNA by RNA polymerase in vitro posed a serious question of specificity until the recent discovery of a new form of RNA polymerase, named RNA polymerase III (and so distinguished from the classic form, RNA polymerase I) which primes DNA synthesis on M13 DNA but not øX174 DNA (*32*). This discrimination is achieved by the presence of a distinctive, small subunit in RNA polymerase III. This small subunit released from RNA polymerase III (by rifampicin or certain procedures used conventionally to isolate RNA polymerase I) can be separated and used to endow RNA polymerase I with the discriminatory capacity of RNA polymerase III. Masking the øX174 template with DNA-unwinding protein is essential for display of specificity by RNA polymerase III. The mechanisms for these effects remain to be elucidated.

The M13 template which appropriates RNA polymerase (either I or III) is clearly distinct from rifampicin-resistant host replicative systems. In this sense the M13 system resembles the replicative systems for plasmids, such as the one for colicinogenic factor El, or the fertility factor, F. Possibly other extrachromosomal DNA's will be found to fall in this group as well.

Table 3. Summary of ϕX174, M13, and G4 replication properties.

Item	Template		
	ϕX174	G4	M13
Initiation			
Rifampicin-sensitive	−	−	+
*dna*B, *dna*C protein i, protein n	+	−	−
*dna*G	+	+	−
RNA polymerase III	−	−	+
Initiation and elongation			
DNA-unwinding protein	+	+	+
Spermidine	+	−	−
Elongation			
DNA polymerase III holoenzyme	+	+	+
Termination			
DNA polymerase I + DNA ligase	+	+	+
Related host replication	Origin	Nascent	Plasmid

Conclusions

Replication is accomplished by multienzyme systems whose operations are usefully considered in respect to three stages of the process: initiation, elongation, and termination.

1) Initiation entails synthesis of a short RNA fragment that serves as primer for the elongation step of DNA synthesis. This stage, probed by SS phage DNA templates, reveals three distinctive and highly specific systems in *E. coli*. The M13 DNA utilizes RNA polymerase in a manner that may reflect how plasmid elements are replicated in the cell. The øX174 DNA does not rely on RNA polymerase, but requires instead five distinctive proteins which may belong to an apparatus for initiating a host chromosome replication cycle at the origin. The G4 DNA, also independent of RNA polymerase, needs simply the *dna*G protein for its distinctive initiation and may thus resemble the system that initiates the replication fragments at the nascent growing fork. In each case it is essential that in vitro the DNA-unwinding protein coat the viral DNA and influence its structure.

2) Elongation is achieved in every case by the multisubunit, holoenzyme form of DNA polymerase III. Copolymerase III*, which is an enzyme subunit, and adenosine triphosphate are required to form a proper complex with the primer template but appear dispensable for the ensuing chain growth by DNA polymerase (*33*).

3) Termination requires excision of the RNA priming fragment, filling of gaps and sealing of interruptions to produce a covalently intact phosphodiester backbone. DNA polymerase I has the capacity for excision and gap-filling and DNA ligase is required for sealing.

What once appeared to be a simple DNA polymerase-mediated conversion of a single-strand to a duplex circle (*34*) is now seen as a complex series of events in which diverse multienzyme systems function. Annoyance with the difficulties in resolving and reconstituting these systems is tempered by the conviction that these are the very systems used by the cell in replicating its chromosome and extrachromosomal elements. Thus, understanding of the regulation of replication events in the cell, their localization at membrane surfaces and integration with cell division, and their coordination with

phage DNA maturation and particle assembly will all be advanced by knowledge of the components of the replicative machinery.

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35. Abbreviations: ATP, GTP, UTP, CTP, adenosine, guanosine, uridine, and cytidine triphosphates; dATP, dGTP, TTP, and dCTP, the corresponding deoxynucleoside triphosphates; dNTP, deoxynucleoside triphosphate; rNTP, ribonucleoside triphosphate; SS, single-stranded DNA; RF, replicative-form DNA.

Copyright: Its Adequacy in Technological Societies

The traditional copyright concept may not be appropriate to knowledge management in a technological society.

Nicholas L. Henry

Marshall McLuhan, communications theorist cum English teacher, has contended that, "in the age of Xerox, every man is a publisher." Rightly or wrongly, McLuhan points up a growing problem of public policy: How to promote both the origination and the accessibility of information in a society increasingly permeated by new information technologies and increasingly dependent on the use of information?

The dilemma is exquisite. On the one hand, the producers of society's knowledge must have economic incentives to produce knowledge. This incentive traditionally has been provided by copyright law. The fundamental thesis of the copyright concept is that the more a knowledge-producer sells of his product, the more he should be compensated. In this fashion, both the individual knowledge-originator and the whole of society will benefit; the producer of information will gain by royalty checks, and the society will gain by acquiring new knowledge. To assure the durability of this arrangement, copyright relies on the device of exclusive licensing; that is, authors, because they invest time and effort, and publishers, because they risk capital, possess the exclusive right to sell the author's work on the open market.

On the other hand, new information technologies have increased and facilitated the accessibility and utility of information dramatically. Technology is the only means that society has for bringing the publication inflation under control; it is the only means that may enable information users to obtain the information that they need, when they need it, an in a parsimonious way, without having to wade through useless data that slow, if not stop, research and knowledgeable decision-making. Moreover, control over the information explosion that technology gives us is becoming increasingly critical in an increasingly complex and interrelated society; if public policies are to be responsive, properly focused, and impactful, then the information on which those policies are founded must be readily accessible and germane to the public problem. In this sense, the reduction of "noise" in the social system assumes a growing importance for public policy-makers if their policies are to have "economy"—that is, be free from spillover effects.

Information technologies, notably photocopying, microphotography, computer-based information storage and retrieval systems, cable television, and microwave communications, may succeed in reducing systemic noise. Information technologies also may succeed in undermining the economic incentive of knowledge-producers to continue producing knowledge. With every man a publisher, traditional publishing houses no longer control the technical means of knowledge production that they once did. Therein lies our dilemma.

It is my purpose in this article to discuss the utility of copyright as a public policy for knowledge management

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